

Ribosomal Resistance to Streptomycin and Spectinomycin in *Neisseria gonorrhoeae*

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A cell-free protein synthesizing system was used to study the mechanism of resistance to streptomycin (Str) and spectinomycin (Spc) in laboratory mutants and clinical isolates of *Neisseria gonorrhoeae*. The 70S ribosomes from sensitive strains were sensitive to the effects of Str and Spc on synthesis directed by several synthetic polynucleotide messengers, whereas 70S ribosomes from resistant strains were resistant to these same effects. In each case, the alteration was localized to the 30S ribosomal subunit by studying antibiotic sensitivities of hybrid 70S ribosomes formed by combining subunits from sensitive and resistant strains. No evidence was found for streptomycin- or spectinomycin-inactivating enzymes.

In the preceding paper we described a cluster of linked genes for antibiotic resistance in *Neisseria gonorrhoeae*, which included loci for resistance to streptomycin (Str) and spectinomycin (Spc) (17). Resistance to these compounds in clinical isolates of other species of bacteria has almost always proven to be due to presence of antibiotic-inactivating enzymes (3). In this communication we report experiments with a cell-free protein synthesizing system, which demonstrate that *str* and *spc* loci in clinical strains as well as laboratory mutants of *N. gonorrhoeae* result in 30S ribosomal resistance to the respective drugs.

MATERIALS AND METHODS

Bacterial strains. The strains are described in the accompanying paper (17). FA5 (*str-1*), FA77 (*spc-1*), and FA19 (*str⁺spc⁺*) are clinical isolates. FA47 (*str-7*) is an ultraviolet-induced mutant of FA19. Whole cells of FA19 are sensitive to 8 μ g of Str and 20 μ g of Spc per ml. FA5 and FA47 are resistant to over 32 mg of Str per ml. FA77 is resistant to 2 mg of Spc per ml.

Media and growth. Most media and conditions for growth were as described (17). Bath cultures were diphasic medium, consisting of a liquid phase of 1,500 ml of GC base broth (Difco) over a base layer of 250 ml of GC base agar (Difco) containing 2% agar (17), in 2.8-liter Fernbach flasks. These were inoculated to an initial density of 10 to 20 Klett units (Klett-Sumner colorimeter, filter no. 54) with clonal type 4 cells (15) harvested from 18-h GC base agar plates. Incubation was at 36 C in 5% CO₂ on a rotary shaker, which resulted in generation times of 60 to 80 min. Cells were harvested at midexponential phase (100 Klett units) by pouring over an equal volume of ice, and, after collection by centrifugation at 4 C, they were washed

once in cold minimal medium Davis (Difco) and stored frozen at -70 C until used. Yield was approximately 1.5 g (wet weight) of cells per liter.

Preparation of ribosomes. Crude S-30 fractions and 70S ribosomes and S-150 fraction were prepared by methods very similar to those previously employed with *Escherichia coli* (18, 20). All preparative procedures were at 0 to 4 C. Five grams of frozen cells was suspended in 10 ml of TNA buffer [30 mM NH₄Cl, 10 mM magnesium acetate, 6 mM β -mercaptoethanol, 10 mM tris(hydroxymethyl)aminomethane(Tris)-hydrochloride, pH 7.5] and were broken by passage through a French Press at 20,000 psi. Crude S-30 fraction was the supernatant remaining from a 30-min, 30,000 \times g centrifugation of the broken cell suspension. Grinding frozen cells with alumina (18) resulted in breakage of most cells, but few ribosomes remained in the S-30 fraction. The 70S ribosomes were prepared the same as the pellet obtained by centrifuging the S-30 fractions for 2 h at 60,000 rpm in a Spinco type 65 rotor. The ribosomes were suspended gently in TAN buffer and were clarified by a 10-min, 10,000 \times g centrifugation. The upper two-thirds of the supernatant remaining after pelleting of the 70S ribosomes was saved as the S-150 fraction.

Salt-washed 70S ribosomes were prepared by suspending the 70S ribosomal pellet in modified TAN buffer containing 1.0 M NH₄Cl with overnight stirring at 0 C (13). The once-washed ribosomes were pelleted by centrifugation, rinsed with TAN, and resuspended in TAN. These and all other subcellular preparations (crude S-30 fractions, 70S ribosomes subunits, and S-150 fraction supernatant enzymes) were dialyzed 4 to 6 h at 0 C against TAN buffer and were stored at -70 C. Ribosomes were stored at concentrations of 20 mg/ml (1.0 optical density units at 260 nm = 60 μ g/ml ribosomes).

Preparation of subunits. Pelleted 70S ribosomes were resuspended in TAS buffer (100 mM NH₄Cl, 1.0

mM magnesium acetate, 20 mM Tris-hydrochloride, pH 7.5, 0.05 mM ethylenediaminetetraacetic acid) (19), and up to 200 optical density units at 260 nm were layered onto linear 5 to 20% sucrose gradients in TAS buffer. After centrifugation for 5.5 h at 27,000 rpm, 4 C, in an SW27 rotor, fractions were collected with an ISCO density-gradient fractionator. Only the initial portions of the 30S peak and the latter portion of the 50S peak were saved to ensure purity. Fractions containing subunits were immediately raised to 10 mM Mg^{2+} and were collected either by precipitation with 70% ethanol (22), or by centrifugation for 10 h at 55,000 rpm at 4 C in a Spinco type 60 rotor. Each batch of subunits was checked for purity by analytical sucrose gradient centrifugation (5 to 20% sucrose, TAS buffer, SW56 rotor, 60 min at 55,000 rpm).

Cell-free synthesis. Conditions used in cell-free synthesis with *E. coli* ribosomes (18) were employed with minor modification. For most experiments, each 100- μ liter reaction mixture contained: 200 μ g of ribosomes; 10 μ liters of S-150 fraction; 60 mM NH_4Cl ; 6 mM β -mercaptoethanol; 50 mM Tris-hydrochloride, pH 7.5; magnesium acetate as indicated; 1 mM adenosine 5'-triphosphate; 0.02 mM guanosine 5'-triphosphate; 5 mM phosphoenolpyruvate; 30 μ g of pyruvate kinase per ml; 20 μ Ci of ^{14}C -labeled amino acid (as indicated) per μ mol, 0.01 mM; 19 other amino acids, 0.05 mM; and synthetic polynucleotide messenger (as indicated), 10 μ g. Addition of polyethylene glycol ^{14}C -labeled 1 to 5% (vol/vol), did not improve efficiency of incorporation. Incubations were at 37 C for 30 min. (Maximal incorporation was achieved by 30 min but was stable over 120 min of incubation.) One drop 0.25% bovine serum albumin was added, and the reactions were stopped by adding 0.5 ml of 6% trichloroacetic acid followed by heating to 90 C for 20 min. The cold trichloroacetic acid-insoluble precipitates were collected by membrane (HA) filtration (Millipore Corp.), and the dried filters were counted in a toluene-based scintillant at efficiencies of approximately 60%.

All experiments were performed at least twice with at least two separate batches of ribosomes, and in each experiment the recorded values were means from duplicate reaction mixtures. Net incorporation into trichloroacetic acid-precipitable material was determined by subtracting background incorporation observed without added messenger.

Chemicals. Polyuridylic acid [poly(U)], polyinosinic acid [poly(I)], polyuridylic-quanylic (1:1) acid [poly(U:G)], and polycytidylic acid [poly(C)] were from Miles. Radioactive amino acids were from New England Nuclear. Adenosine 5'-triphosphate, guanosine 5'-triphosphate, phosphoenolpyruvate, and pyruvate kinase were from Sigma. Ribonuclease-free sucrose (density gradient grade) was from Schwarz-Mann. Spectinomycin was from Upjohn; streptomycin was from Pfizer. Other reagents were of highest available purity.

Assay for antibiotic-inactivating enzymes. Sensitive and resistant whole cells, and cell-free extracts freshly prepared from exponential-phase cells either by sonic treatment or passage through a French press,

were incubated for 60 min at 37 C in a buffer containing 20 mM adenosine 5'-triphosphate, 0.04 mM acetyl coenzyme A, 10 mM sodium acetate, 50 mM NH_4Cl , 10 mM β -mercaptoethanol, 100 mM Tris-hydrochloride, pH 7.5, and 10 mM $MgCl_2$ plus 100 μ g of either spectinomycin or streptomycin per ml. Protein concentration in the 1.0-ml reaction mixture was 5 mg/ml. Similar incubations without added cells were employed as controls. At the beginning and end of the incubation, samples of 0.020 ml were removed to wells in antibiotic II agar (Difco) containing *Sarcina lutea*; antibiotic activities were determined by measuring sizes of zones of inhibition after overnight incubation at 37 C, as compared to a series of standards made up in the same buffer (2).

RESULTS

Definition of the system. Crude S-30 fractions or partially purified 70S ribosomes plus S-150 fraction enzymes were active in cell-free protein synthesis directed by several synthetic polynucleotide messengers, including poly(U)-directed incorporation of [^{14}C]phenylalanine (Phe), poly(U:G)-directed (1:1) incorporation of [^{14}C]valine (Val), and poly(I)-directed incorporation of [^{14}C]Val. Each of these messengers stimulated incorporation at least 10-fold above that observed without added messenger. Little activity was noted in poly(C)-directed incorporation of [^{14}C]proline. Incorporation of [^{14}C]Val or [^{14}C]Phe directed by residual endogenous (natural) messenger ribonucleic acid was usually low. Preincubation of ribosomes for 10 min in 50 mM Tris-hydrochloride (pH 7.5), 0.02 mM guanosine 5'-triphosphate, 5 mM phosphoenolpyruvate, and 30 μ g of pyruvate kinase per ml at 36 C (18) prior to adding synthetic messenger resulted in twofold or greater decreases in endogenous messenger ribonucleic acid activity, but equal or greater losses in activity of ribosomes in synthesis directed by poly(I) or poly(U) (data not shown). For this reason, ribosomes were not routinely preincubated. Optimal Mg^{2+} concentrations for synthesis directed by poly(U) or poly(U:G) (1:1) were 10 to 15 mM, and for poly(I) were 25 to 30 mM.

Activity of the cell-free system was linearly dependent on ribosome concentration, up to 1,000 μ g/ml (100 μ g per each 100- μ liter reaction mixture) (Fig. 1). Activity of 70S ribosomes purified once by centrifugation was partially dependent upon addition of S-150 fraction supernatant enzymes, but addition of excess S-150 fraction resulted in marked inhibition of incorporation (Fig. 2). Similar inhibition by S-150 fraction was noted in poly(I)-directed [^{14}C]Val incorporation. No explanation for this effect was apparent.

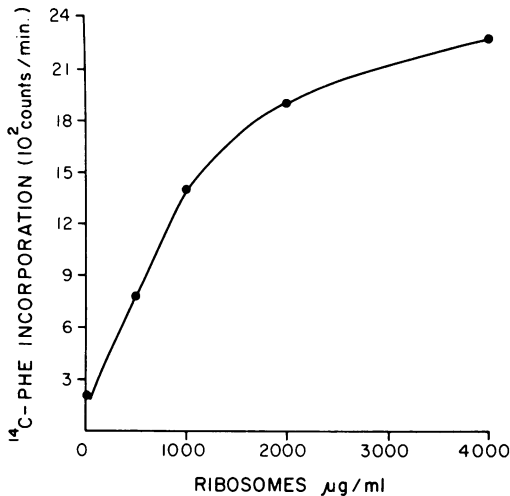


FIG. 1. Effect of concentration of 70S ribosomes from strain FA5 on incorporation of [^{14}C]phenylalanine directed by poly(U) at 12.5 mM Mg^{2+} . Other conditions as described.

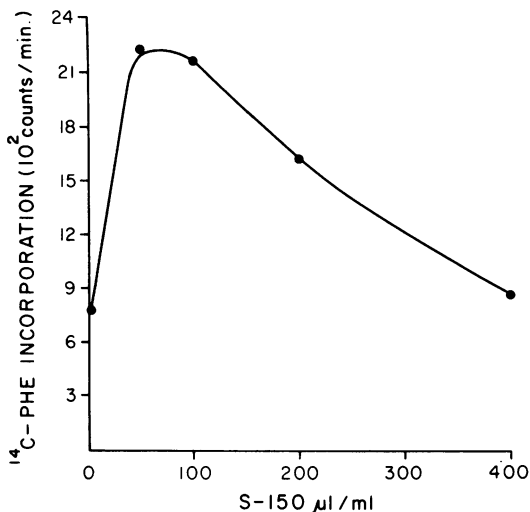


FIG. 2. Effect of supernatant enzyme fraction (S-150) from strain FA5 on incorporation of [^{14}C]phenylalanine directed by poly(U) at 12.5 mM Mg^{2+} . The 70S ribosomes were from FA5. Other conditions as described.

Ribosomes from *E. coli* K-12 strain MX129 were considerably less active with heterologous gonococcal S-150 fraction than with homologous preparations from *E. coli*. Gonococcal ribosomes were also much less active with *E. coli* S-150 fraction than with gonococcal S-150 fraction (Table 1). Use of MS-2 ribonucleic acid (12) as messenger at 10 mM Mg^{2+} resulted in no incorporation of [^{14}C]Val in the gonococcal system, although it was quite active in stimu-

lating incorporation under similar conditions with *E. coli* ribosomes (data not shown). These observations suggested species specificity of one or several components of the translational apparatus in these organisms, but precise definition of which factor(s) were involved was not attempted.

Ribosomal streptomycin resistance. The 70S ribosomes were prepared from streptomycin sensitive (Str^s) strain FA19, and streptomycin resistant (Str^r) strains FA47 and FA5. These were used with S-150 fraction from Str^s strain FA19 in cell-free synthesis of [^{14}C]poly Phe directed by poly(U). Ribosomes from the Str^s strain were inhibited approximately 30% by addition of 10 to 100 μg of Str per ml, but ribosomes from the two Str^r strains were inhibited only 0 to 8% (Fig. 3). The inhibition of the Str^s ribosomes was approximately equal to that observed under similar conditions with Str^s *E. coli* ribosomes (5). Results were nearly identical when crude S-30 fractions were used instead of 70S ribosomes, and when sensitivity to Str of poly(U:G) (1:1) directed incorporation of [^{14}C]Val was studied at 10 mM Mg^{2+} .

As an additional test, the ability of Str to cause translational infidelity (misreading) was measured in a poly(U)-directed system. In *E. coli*, it has previously been established that Str causes misreading of synthesis directed by several polynucleotide messengers, resulting in incorporation of "incorrect" amino acids. In poly(U)-directed synthesis, Str causes increased incorporation of isoleucine (Ile, the "incorrect" amino acid) but decreased incorporation of Phe (the "correct" amino acid) (7). This effect has been seen with Str^s but not Str^r ribosomes (7).

TABLE 1. Requirement of gonococcal and *E. coli* ribosomes for homologous supernatant factors

70S Ribosomes ^a	S-150 ^a	Poly(U)-directed [^{14}C]Phe incorporation ^b	
		Net counts/min	Relative activity (%)
GC	GC	2,201	
GC	<i>E. coli</i>	464	21
<i>E. coli</i>	<i>E. coli</i>	3,425	
<i>E. coli</i>	GC	1,212	38

^a The 70S ribosomes and S-150 fraction from *N. gonorrhoeae* strain FA19 (GC) and *E. coli* strain MX129, in amounts (respectively) of 200 μg and 10 μl iters per each 100- μl iter reaction mixture.

^b Incorporation at 12.5 mM Mg^{2+} .

^c Activity of ribosomes with heterologous S-150 fraction/activity with homologous S-150 fraction.

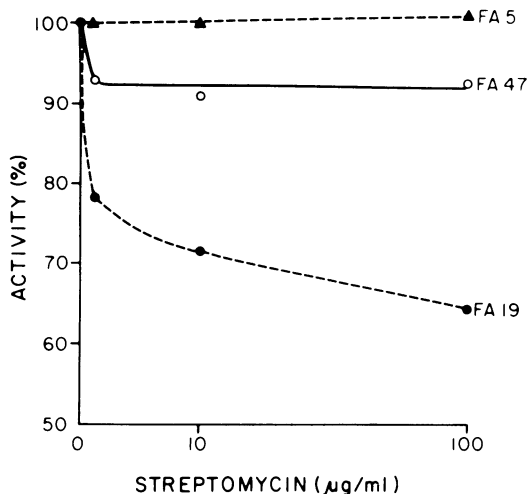


FIG. 3. Inhibition of poly(U)-directed [^{14}C]phenylalanine incorporation by streptomycin. The 70S ribosomes were from FA5 (*str*⁻), FA47 (*str*⁻), and FA19 (*str*⁺). The S-150 fraction was from FA19. Mg^{2+} = 10 mM. Other conditions as described.

Similar results were obtained with ribosome prepared from *Str*⁻ and *Str*⁺ strains of *N. gonorrhoeae* (Fig. 4). Addition of *Str* in concentrations up to 100 $\mu\text{g}/\text{ml}$ resulted in no misreading of poly(U) by 70S ribosomes from *Str*⁺ strains FA5 and FA47, but *Str* caused markedly increased misreading (expressed as the ratio of incorporation of Ile/Phe) with ribosomes from *Str*⁻ strain FA19. Results were unchanged by use of S-150 fraction enzymes from either *Str*⁻ or *Str*⁺ strains, or by use of ribosomes washed once in 1 M NH_4Cl . Thus, resistance to *Str* in both a laboratory-derived *Str*⁺ mutant (FA47) and a clinically isolated *Str*⁺ strain (FA5) was apparently due to ribosomal resistance to the effect of the drug.

Ribosomal spectinomycin resistance. Gonococcal ribosomes (S-30 fraction or 70S ribosomes plus S-150 fraction) were quite active in synthesis directed by the Spc-inhibitable messengers poly(U:G) (1:1) or poly(I) (1). As is the case with *E. coli* ribosomes (1, 9), activity of poly(I) was dependent on high concentrations of divalent cation, reaching a maximum at 25 to 30 mM Mg^{2+} . Spc had no inhibitory effect on poly(U)-directed incorporation of [^{14}C]Phe, as previously reported (1).

When 70S ribosomes from Spc⁺ strain FA77 were compared to those from Spc⁻ strain FA19, there was a marked inhibitory effect of Spc on ribosomes from the Spc⁻ strain but little effect on ribosomes from the Spc⁺ strain (Table 2). Washing the ribosomes once in 1 M NH_4Cl

decreased the sensitivity to Spc of ribosomes from both strains and resulted in slight stimulation by Spc of incorporation with Spc⁺ ribosomes (Table 2). The inhibitory effect of Spc was quite concentration-dependent, with maximal inhibition only at 100 to 300 $\mu\text{g}/\text{ml}$ (Fig. 5). Results similar to Table 2 and Fig. 5 were obtained in incorporations directed by poly(U:G) at 10 mM Mg^{2+} . Again, no differences in the results were observed when S-150 fractions from either a Spc⁻ or a Spc⁺ strain were used with the 70S ribosomes (data not shown).

The 30S subunit determines *Str*⁺. Earlier experiments with *E. coli* have established that ribosomal resistance to *Str* or Spc is due to

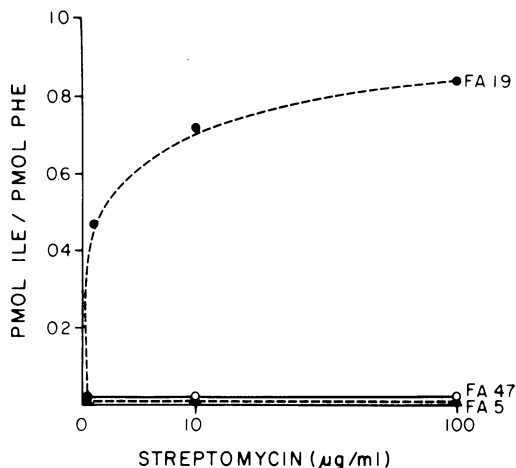


FIG. 4. Effect of streptomycin on misreading of poly(U), at 10 mM Mg^{2+} , as measured by the ratio of incorporation of [^{14}C]isoleucine (Ile) to [^{14}C]phenylalanine (Phe). The 70S ribosomes were from FA5 (*str*⁻), FA47 (*str*⁻), and FA19 (*str*⁺). The S-150 fraction was from FA19. Other conditions as described.

TABLE 2. Effect of salt washing 70S ribosomes on their resistance to spectinomycin

70S Ribosomes ^a	Poly(I)-directed [^{14}C]Val incorporation ^b (net counts/min)		
	- Spc	+ Spc (100 $\mu\text{g}/\text{ml}$)	%
S—unwashed	1,952	104	5.3
S—washed	2,158	260	12.0
R—unwashed	2,433	1,768	72.0
R—washed	2,024	2,215	110.0

^a S, Ribosomes from Spc⁻ strain FA19; R, ribosomes from Spc⁺ strain FA77. Ribosomes were washed once in 1.0 M NH_4Cl as described.

^b Incorporation at 28 mM Mg^{2+} .

particular structural alterations in the 30S ribosomal subunit (4-6, 10, 11, 16). It therefore seemed likely that if gonococcal resistance to these drugs were truly ribosomal, rather than due to presence of antibiotic-inactivating enzymes which were firmly bound to 70S ribosomes, it should be possible to demonstrate subunit specificity to resistance to each drug. Accordingly, hybrid ribosomes were formed in vitro with various combinations of subunits prepared from Str^s and Str^r strains, and sensitivity to inhibition by Str of poly(U)-directed [¹⁴C]Phe incorporation was measured. The 30S subunit determined the streptomycin sensitivity or resistance of the hybrid ribosome (Table 3). Similar results were obtained with a second preparation of subunits from the same strains.

When the same type of experiment was undertaken with ribosomes from Spc^s and Spc^r strains (FA19 and FA77, respectively), considerable difficulty was encountered in preparing subunits which retained their activity in synthesis directed by either poly(I) or poly(U:G). This necessitated careful reexamination of factors which affected activity of subunits (Table 4). Activity was markedly diminished by use of the convenient technique of ethanol precipitation of subunits from sucrose gradient fractions (22), or by repeated cycles of purification on sucrose gradients. Activity was affected less if subunits were collected from sucrose gradient fractions by ultracentrifugation. All preparations of subunits required higher concentrations of Mg²⁺ than the original 70S ribosomes for optimal activity, either with poly(U:G) (Table

TABLE 3. Resistance of naturally occurring Str^r *N. gonorrhoeae* mutant is due to altered 30S ribosome

Ribosomal subunits ^a		Poly(U)-directed [¹⁴ C]Phe incorporation ^b (net counts/min)		
30S	50S	- Str	+ Str (100 μg/ml)	%
S	S	2,623	2,169	82
S	R	1,239	796	64
R	S	2,683	2,840	106
R	R	1,411	1,468	104

^a Purified once on sucrose gradients and concentrated by ethanol precipitation, as per Materials and Methods. Each reaction mixture contained 60 μg of 30S subunits and 120 μg of 50S subunits. S, Ribosomes from Str^s strain FA19; R, ribosomes from Str^r strain FA5.

^b Incorporation at 15 mM Mg²⁺.

TABLE 4. Effect of method of preparation on activity of gonococcal ribosomal subunits

Source	Ribosome ^a	Poly(U:G)-directed [¹⁴ C]Val incorporation (net counts/min)		
		10 mM Mg ²⁺	15 mM Mg ²⁺	20 mM Mg ²⁺
FA19	70S	3,350	1,870	820
	30S + 50S (1×, pellet)	512	568	490
	30S + 50S (1×, ETOH)	83	218	199
	30S + 50S (2×, ETOH)	3	90	57
FA77	70S	1,690	1,438	1,100
	30S + 50S (1×, pellet)	113	307	384

^a Subunits were purified on sucrose gradients as described. 1×, One cycle of sucrose gradient centrifugation; 2×, two cycles of sucrose gradient centrifugation; pellet, concentrated from sucrose by centrifugation for 10 h, 55,000 rpm; ETOH, concentrated from sucrose by precipitation with 70% ethanol. Each reaction mixture contained 60 μg of 30S subunits and 120 μg of 50S subunits.

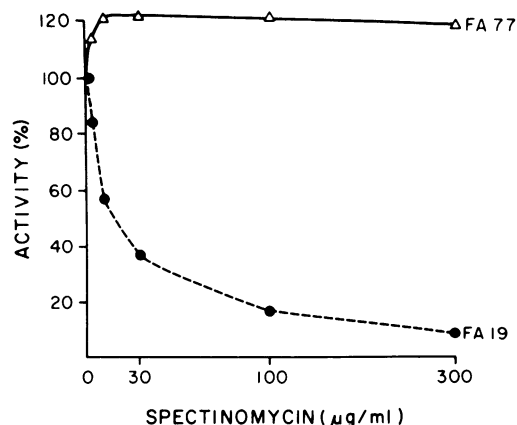


FIG. 5. Inhibition by spectinomycin of poly(I)-directed incorporation of [¹⁴C]valine at 27.5 mM Mg²⁺. The 70S ribosomes were from FA77 (spc-1) and FA19 (spc⁺). The S-150 fraction was from FA19. Other conditions as described.

4) or poly(I) (not shown). Activity of subunits in poly(U)-directed incorporations exhibited similar characteristics, except relatively less loss of activity was noted. Thus, ribosomal subunits from the studied strains of *N. gonorrhoeae* were considerably more unstable than similarly prepared subunits from *E. coli* K-12 strains (Y. Ikeya, G. Foster, and P. F. Sparling, unpublished observations).

The 30S subunit determines Spc^r. Subunits prepared by a single cycle of sucrose gradient centrifugation, and subsequently concentrated by pelleting in the ultracentrifuge, were sufficiently active in poly(U:G)- or poly(I)-directed synthesis to permit determination of which

subunit was altered by the mutation to Spc^R . Resistance to spectinomycin was clearly determined by the 30S and not the 50S subunit (Table 5).

Inactivating enzymes. Whole cells and cell extracts of Str^S Spc^S strain FA19, Str^R strain FA5, and Spc^R strain FA77 were incubated with Str or Spc for 60 min under conditions (see above) expected to demonstrate presence of known enzymes in enteric bacteria which inactivate these antibiotics (3). No loss of biologic activity of either drug was noted under these conditions with any of the three tested strains. Since the bioassay was able to detect differences as small as 10 μ g of either drug per ml (authors unpublished experiments), and since the resistant strains were capable of growing in the presence of at least 2,000 μ g of the respective drug per ml, it seemed unlikely that resistance was due to antibiotic-inactivating enzymes.

DISCUSSION

We have shown that mutations to high-level resistance to either streptomycin or spectinomycin in clinical isolates and in vitro mutants of *N. gonorrhoeae* are associated with 30S ribosomal resistance to the respective drug. It is quite unlikely that the observed results were due to antibiotic-inactivating enzymes. If such enzymes were present, one would have to postulate that they were resistant to removal from the ribosome either by washing once in 1.0 M NH_4Cl or during sucrose gradient centrifugation, and that the enzymes were specifically bound to the 30S subunit. Moreover, we failed to find evidence for Spc - or Str -inactivating enzymes in whole cells or cell extracts. A much more likely explanation is that the *str-1*, *str-7*, and *spc-1* gene products affect the structure of

the 30S ribosome, thereby rendering resistant the target site of action of these drugs.

In the preceding paper, we showed that these *str* and *spc* loci are linked by transformation and are located in a cluster of genes for antibiotic resistance (17), which is similar to the reported clusters of structural genes for ribosomal proteins in *E. coli* and *Bacillus subtilis* (8, 10, 11). If the genetic analogies are correct, the studied gonococcal *str* and *spc* loci probably are structural genes for ribosomal proteins. Since loci for resistance to tetracycline and chloramphenicol map between the *spc-1* and *str-7* loci (17), and these drugs also act on the ribosome (21), it is likely that the *tet* and *chl* loci also specify ribosomal proteins.

Resistance in clinical isolates of bacteria to antibiotics which act on the ribosome is usually due to genes, often carried on plasmids, for antibiotic-inactivating enzymes (3), or which decrease uptake of drug through the cell envelope (14). The only clearly defined exception is high-level resistance of the enterococcus to streptomycin, which was shown by Zimmermann, Moellering, and Weinberg (23) to be due to 30S ribosomal resistance to *str*. Thus, the gonococcus is a relative exception to the general rule that ribosomal mutants are rare in nature. In the gonococcus, the evidence strongly suggests that mutations of the ribosome are frequently the basis for resistance to both streptomycin and spectinomycin.

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LITERATURE CITED

- Anderson, P., J. A. Davies, and B. D. Davis. 1967. Effect of spectinomycin on polypeptide synthesis in extracts of *Escherichia coli*. *J. Mol. Biol.* **29**:203-215.
- Barza, M., M. G. Bergeron, J. Bruschi, and L. Weinstein. 1973. Selective filtration of antibiotics through colloid membranes. *Antimicrob. Ag. Chemother.* **4**:337-342.
- Benveniste, R., and J. Davies. 1973. Mechanisms of antibiotic resistance in bacteria. *Annu. Rev. Biochem.* **42**:471-506.
- Bollen, A., J. Davies, M. Ozaki, and S. Mizushima. 1969. Ribosomal protein conferring sensitivity to the antibiotic spectinomycin in *Escherichia coli*. *Science* **165**:85-86.
- Davies, J. 1964. Studies on the ribosomes of streptomycin-sensitive and resistant strains of *Escherichia coli*. *Proc. Nat. Acad. Sci. U.S.A.* **51**:659-664.
- Davies, J., P. Anderson, and B. D. Davis. 1965. Inhibition of protein synthesis by spectinomycin. *Science* **149**:1096-1098.
- Davies, J., W. Gilbert, and L. Gorini. 1964. Strep-

TABLE 5. Resistance of naturally occurring Spc^R *N. gonorrhoeae* mutant is due to altered 30S ribosome

Ribosomal subunits ^a		Poly(U:G)-directed [¹⁴ C]Val incorporation ^b (net counts/min)		
30S	50S	- Spc	+ Spc (3 μ g/ml)	%
S	S	266	8	3
S	R	263	4	1
R	S	460	183	39
R	R	373	180	48

^a Purified once on sucrose gradients and concentrated by ultracentrifugation as described. Each reaction mixture contained 60 μ g of 30S subunits and 120 μ g of 50S subunits. S, Ribosomes from Spc^S strain FA19; R, ribosomes from Spc^R strain FA77.

^b Incorporation at 13 mM Mg^{2+} .

- tomycin, suppression and the code. Proc. Nat. Acad. Sci. U.S.A. **51**:883-890.
8. Davies, J., and M. Nomura. 1972. The genetics of bacterial ribosomes. Annu. Rev. Genet. **6**:203-234.
 9. Davis, B. D., P. Anderson, and P. F. Sparling. 1973. Pairing of inosine with adenosine in codon position two in the translation of polyinosinic acid. J. Mol. Biol. **76**:223-232.
 10. Funatsu, G., E. Schlitz, and H. G. Wittmann. 1971. Ribosomal proteins. XXVII. Localization of the amino acid exchanges in protein S5 from two *Escherichia coli* mutants resistant to spectinomycin. Mol. Gen. Genet. **114**:106-111.
 11. Funatsu, G., and H. C. Wittmann. 1972. Ribosomal proteins. XXXIII. Location of amino-acid replacements in protein S12 isolated from *Escherichia coli* mutants resistant to streptomycin. J. Mol. Biol. **68**:547-550.
 12. Gesteland, R. F., and H. Boedtker. 1964. Some physical properties of bacteriophage R17 and its ribonucleic acid. J. Mol. Biol. **8**:496-507.
 13. Hershey, J. W. B., E. Remold-O'Donnell, D. Kolakofsky, K. F. Dewey, and R. E. Thach. 1971. Isolation and purification of initiation factors F1 and F2, p. 235-247. In K. Moldave and L. Grossman (ed.), Methods in enzymology, vol. 20. Academic Press Inc., New York.
 14. Izaki, K., and K. Arima. 1963. Disappearance of oxytetracycline accumulation in the cells of multiple drug-resistant *Escherichia coli*. Nature (London) **200**:384-385.
 15. Kellogg, D. S., Jr., W. L. Peacock, Jr., W. E. Deacon, L. Brown, and C. I. Pirkle. 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. J. Bacteriol. **85**:1274-1279.
 16. Nomura, M. 1970. Bacterial ribosome. Bacteriol. Rev. **34**:228-277.
 17. Sarubbi, F. A., Jr., E. Blackman, and P. F. Sparling. Genetic mapping of linked antibiotic resistance loci in *Neisseria gonorrhoeae*. J. Bacteriol. **120**:1284-1292.
 18. Sparling, P. F., J. B. Modolell, Y. Takeda, and B. D. Davis. 1968. Ribosomes from *E. coli* merodiploids heterozygous for sensitivity and resistance to spectinomycin and streptomycin. J. Mol. Biol. **37**:407-421.
 19. Staehelin, T., and D. R. Maglott. 1971. Preparation of *Escherichia coli* ribosomal subunits active in polypeptide synthesis, p. 449-456. In K. Moldave and L. Grossman (ed.), Methods in enzymology, vol. 20. Academic Press Inc., New York.
 20. Traub, P., S. Mizushima, C. V. Lowry, and M. Nomura. 1971. Reconstitution of ribosomes from subribosomal components, p. 391-407. In K. Moldave and L. Grossman (ed.), Methods in enzymology, vol. 20. Academic Press Inc., New York.
 21. Weisblum, B., and J. Davies. 1968. Antibiotic inhibitors of the bacterial ribosome. Bacteriol. Rev. **32**:493-528.
 22. Zimmermann, R. A., R. T. Garvin, and L. Gorini. 1971. Alteration of a 30S ribosomal protein accompanying the *ram* mutation in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **68**:2263-2267.
 23. Zimmermann, R. A., R. C. Moellering, Jr., and A. N. Weinberg. 1971. Mechanism of resistance to antibiotic synergism in enterococci. J. Bacteriol. **105**:873-879.